dissolving at a pH between about 8 and 9, and carrying out the separating of the mevinolin at a pH of between 4.5 and 2.

D3

13. (Amended) The process of claim 11, wherein the recovering of the mevinolin product is carried out at a pH between about 2 and about 2.2.

REMARKS

Claims 4, 6 and 11-16 are in the application.

The amendments presented above are believed to overcome the 35 U.S.C. 112, second paragraph rejections. Reconsideration and withdrawal thereof are requested..

The examiner has maintained the previous rejection of claim 11 under 35 U.S.C. 102(e) over U.S. Patent no. 5,403,728 to Jekkel et al. ("Jekkel"), and of claims 4-6 and 11-16 under 35 U.S.C. 103(a) over Jekkel taken with Nakamura et al. ("Nakamura"). The applicant respectfully traverses. Both rejections will be treated together.

Applicant again submits that, although no pH value is expressly stated in the Jekkel specification for the isolation of the active ingredient from the fungal cells, one skilled in the art is able to discern that Jekkel discloses using a pH of 10-10.2 for this step. The examiner's attention is drawn to the Jekkel specification at col. 7, lines 24-27, which state that a 2N sodium hydroxide or triethylamine solution can be used in place of ammonium hydroxide, and to col. 7, lines 40-42, which describe the subsequent acidification step. The examiner's attention is also drawn to Jekkel's Example 2 (col. 10, line 15-col. 11, line 51) from which one skilled in the art can readily calculate the pH used to remove the active ingredient from the cells. In Example 2, 300 mL of 25% ammonium hydroxide (pH 12-13) were added to 9.5 L of fermentation broth (col. 10, lines 36-41), resulting in a final concentration of about 0.35 M ammonium hydroxide. Since the fermentation broth contained only 2 g of acid (KH_2PO_4, col. 10, line 29), which resulted in a final concentration of about 0.015 M acid, ammonium hydroxide is present in large excess. Therefore, the resulting pH of the fermentation broth in this step is between 10 and 10.2, and not between 7.5-10.

The isolation and purification steps of the present invention are different from those recited in Jekkel, and therefore, Jekkel cannot anticipate the claimed invention. In Example 2 of Jekkel, the mevinolin is isolated from filtered fermentation liquor after adjustment to pH 10-10.2 by ion exchange chromatography (col. 10, lines 47-52), and not by precipitation by acidification, as in the present invention. Thus, in Jekkel, adjustment of the pH to 10 is not followed directly by acid treatment. Rather, there is an ion

exchange step between the two pH adjustment steps. Furthermore, after isolation by ion exchange chromatography, acidification by the addition of 15% sulfuric acid is performed in Jekkel in order to convert the mevinolin to the lactone form (col. 10, line 64-col. 11, line 3). Thus, in Jekkel, acidification does not cause the mevinolin to separate from the liquid so that it can simply be filtered out. In contrast, in the present invention, after isolation from the fungal cells, mevinolin is immediately separated from the fermentation broth by adjustment of the pH to about 4.5-1.

Furthermore, separation of mevinolin from the fermentation liquor at the pH values of the claimed invention is not rendered obvious by the cited references. The solubility of an active ingredient such as mevinolin is pH dependent because the molecular structure is affected by the presence of acidic or basic conditions. Therefore, there is an optimal pH for extraction of the material from an aqueous layer into an organic layer. However, this does not render obvious the optimal pH of precipitation, since the active ingredient is unlikely to precipitate from the aqueous layer at the pH value that is optimal for extraction from that layer. If this were the case, then there would be no need for extraction. Separation by precipitation is particularly unexpected where the fermentation liquor is dilute, is contaminated with other substances, or is filtered. In addition, one skilled in the art is unlikely to recover the active ingredient through direct precipitation during the workup of a fermentation liquor because, even if there is precipitation, the active ingredient would still be very difficult to purify.

Applicant re-emphasizes that a pH of 7.5-10 is required for the re-dissolution of mevinolin from the fungal cells because in the absence of this step, separation may not be achieved at pH 4.5-1, either because there is no precipitation at all, or there is very little precipitation and the precipitate is too contaminated to purify the active ingredient in sufficient yield.

Furthermore, independent claims 11 and 12 recite the process as "consisting essentially of" the recited steps, which restricts the invention to the specified conditions and to others that do not materially affect the fundamental characteristics of the claimed invention. The claims exclude unspecified process steps, such as using an ion exchange medium, as disclosed in Jekkel. The direct precipitation of the active ingredient is inherent in the claimed process of the instant invention.

To substantiate that the present invention is a different, simpler and better process than that disclosed in Jekkel and that the present invention is carried out at a different pH, attached to this response is a Rule 132 Declaration filed in the parent application. The declaration is by Dr. Kálmán Pólya, who has obtained a Doctorate in Chemistry, who has extensive experience in fermentation and biotechnology research, and who has taken part in and supervised the development of processes for producing and purifying mevinolin, cyclosporin and other active ingredients. This declaration compares two side-by-side preparations of mevinolin, one carried out according to Example 1 of Jekkel, and the other carried out according to the instant invention. Both processes were carried out

under Dr. Pólya's supervision. This declaration shows data indicating that the mevinolin isolated according to the process of the present invention has substantially fewer contaminants than the mevinolin isolated according to the process disclosed in Jekkel. Furthermore, the declaration states that there were additional contaminants in the sample isolated according to Jekkel, as shown by additional peaks that appeared in the HPLC chromatogram of the Jekkel sample, but not in that of the sample isolated according to the present invention. Therefore, the declaration states that the process of the present invention results in a surprisingly and unexpectedly pure mevinolin product. The declaration also shows a side-by-side comparison of the steps in the processes of (1) U.S. Patent 4,342,767, Example 6; (2) Jekkel, Example 1; (3) Jekkel, Example 2; and (4) the present invention. This comparison indicates that the process of the present invention is simpler in that it requires fewer steps—for example, there is no need for an ion exchange chromatography step. Therefore, this declaration states that the process of the present invention not only results in a much purer product in higher yield, but also accomplishes this in a much simpler way than either the '767 patent or Jekkel. The declaration also states that, although it was known from Jekkel that mevinolin could be extracted from the fermentation liquor at an acidic pH, this reference does not suggest to one skilled in the art that mevinolin can be precipitated and separated by filtration from the fermentation liquor at an acidic pH. Thus, in view of Jekkel, one skilled in the art would not expect to be able to recover relatively pure mevinolin in relatively high yield by filtering it from the fermentation liquor at an acidic pH, especially where the mevinolin is in a very dilute solution. The declaration also states that there is no fermentation process known to the declarant where the active ingredient can be directly removed from the fermentation liquor, except where the active ingredient was removed in very low yield or was too contaminated to purify economically.

The examiner has also maintained the previous rejection of claims 4, 6 and 11-16 under 35 U.S.C. 103(a) over Nakamura et al. taken with Tsujita et al. and Endo et al. applicant respectfully traverse this rejection for the reasons stated above.

In view of the foregoing, reconsideration of the outstanding rejections, and the allowance of claims 4, 6 and 11-16, is respectfully urged.

Respectfully submitted,

Address for Customer No. 23622

Gabriel P. Katona, attorney of record

It is hereby certified that this is being mailed on June 11, 2002

COMPARISON COPY OF CLAIM AMENDMENTS

4. The process of claim [10] 12, wherein the separating of the mevinolin is carried out at a pH between about 2.2 and about 2.

6. The process of claim [5] 15, wherein said additive is ethanol, or ethylene glycol.

12. In a process for preparing mevinolin by fermentation of a culture medium, which includes dissolving mevinolin formed into the culture medium obtained by cultivation of at least one of an *Aspergillus terreus* and *Aspergillus obscurus* strain, and separating the strain from the culture medium to obtain a separated culture medium, separating the mevinolin from the separated culture medium, and recovering the [end] mevinolin product, the improvement which consists essentially of carrying out the dissolving at a pH between about 8 and 9, and carrying out the separating of the mevinolin at a pH of between 4.5 and 2.

13. The process of claim 11, wherein the [separating] recovering of the mevinolin product is carried out at a pH between about 2 and about 2.2.